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DEVELOPMENT OF ASCUS AND SPORE FORMATION IN ASCOMYCETES.

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No. 4. — DEVELOPMENT OF ASCUS AND SPORE FORMATION IN ASCOMYCETES.[1]

BY J. HORACE FAULL.

De Bary ('63, '84) was the first contributor to our knowledge of the cytology of the ascus. His investigations showed that the comparatively young ascus was uninucleate and that it continued to be so until its maximum size was reached; that three successive nuclear divisions followed; that simultaneously about each of the eight resultant nuclei there gathered a rounded mass of protoplasm differing from the surrounding protoplasm, the epiplasm, by its greater transparency, and bounded off from the latter by a delicate line; and finally that these spore initials acquired an exospore and matured at the expense of the epiplasm.

Strasburger ('80) and Schmitz confirmed De Bary's observations in a number of forms, and Gjurasin ('93) not only did the same in *Peziza vesiculosa* Bull., but by the aid of a more modern technique followed with much greater minuteness the processes within the ascus. Gjurasin discovered that the nuclear divisions were karyokinetic, that there were well marked asters, though centrosomes could not be demonstrated, that the anaphase stage was reached before the nuclear wall disappeared, and that the nucleolus remained until the daughter nuclei were formed. Of greatest interest were his observations on the last nuclear division. The third set, unlike the first two sets of spindles, was placed with the long axes at right angles to the long axis of the cell. The asters of this generation persisted much later than those of the two former. During the final stages of division the spindles elongated considerably, bringing the asters near the wall of the ascus. He figured the rays as continuing in the same position as when attached to the spindles, though disconnected from the nuclei and folded inwards over the latter like the ribs of an opened umbrella.

Dangeard's ('94) attention was fixed on the young ascus. He described the origin of the asci in *Peziza vesiculosa*, *Borrera ciliaris*, and some two or three other forms. The first indication of the

[1] Contributions from the Cryptogamic laboratory of Harvard university.— LXI.

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advent of an ascus according to this investigator, was the appearance of four nuclei in a recurved tip of an ascogenous hypha. Next, by means of transversely placed walls, these nuclei were separated from one another so that one lay in the end, two in the second cell, the curved portion of the hypha, and the fourth in the third cell. The ascus grew out from the second cell, the two nuclei of which fused, resulting in a uninucleate condition of the ascus, the earliest stage observed by De Bary. Dangeard at first maintained that the ascus grew out from the point of junction of two hyphal threads, which he supposed to be male and female organs respectively. Subsequently he discovered that there was no such fusion, but that the phenomena are such as I have just described.

Harper (:00) has verified Dangeard's observations in *Pyronema confluens*. He has shown further that there are at first two nuclei in the recurved tip of an ascogenous hypha, that these divide mitotically and synchronously to form the four observed by Dangeard, and that one of each pair of daughter nuclei is relegated to the young ascus. Harper has also confirmed the work of Gjurasin, but his most important work began where Gjurasin's left off. His main problem has been to determine the exact process involved in the delimitation of the plasma of each spore. He has investigated the origin of the spores in *Erysiphe communis*, *Peziza vesiculosa*, *Ascobolus furfuraceus* (Harper, '97) *Lachnea scutellata* (Harper, '99), and *Pyronema confluens* (Harper, :00), and has concluded that the process is practically the same for all of the Ascomycetes.

According to Harper this process is a distinctly unique one. The first step consists in the formation of a beak by each nucleus. This beak bears at its extremity the persistent centrosome and astral rays. The astral rays next swing outwards and down, the uppermost coming into contact laterally and fusing into a thin membrane. At this stage as far as relative positions are concerned, the membrane bears the same relation to the nuclear beak that the cover of an opened umbrella does to the rod that supports it. This "kinoplasmic" membrane continues to grow downward. Eventually it curves inward, the edges meet at a focal point, and the process of delimitation is complete. There has been no aggregation of protoplasm about the nucleus, but the membrane of fused astral rays, the plasma membrane of the young spore, literally cuts the spore plasma out of a homogeneous mass of protoplasm.

Berlese ('99) investigated the sporogenesis of *Tuber brumale*. His results in the main corroborated those obtained by Gjurasin and Harper. He expressed the opinion that the astral rays determine the formation of the plasma membrane of the young spore.

The only other work to be noted in this connection is that of Errera and Guilliermond. Errera ('82) demonstrated for the first time the presence of glycogen in the epiplasm. It occurs in a semi-fluid, amorphous, strongly refractive form, the reactions of which are identical with those of animal glycogen and the function of which is likewise taken to be nutritive. Guilliermond (: 03, : 03b) has recently pointed out the presence of certain granules in the asci of *Ascobolus marginatus*, the Aleurieae, the Helvellineae, etc., and in the spores of *Peziza coccinea*, which he, too, considers to be reserve matter, the same as the "corpuscules métachromatiques" or "granulations basophyles" described by him (: 02) in the yeasts and moulds, and very like, if not the same as, Bütschli's "grains rouges" and Babe's "corpuscules métachromatiques" found by them in bacteria and the Cyanophyceae.

From the foregoing it is quite evident that the origin and cytology of the ascus are known in but a small number of forms, and on these are based generalizations for the entire group. Briefly, it is considered that the ascus is an outgrowth of the penultimate cell of the recurved tip of an ascogenous hypha, that it at first contains two nuclei which later on fuse, that there follows a series of mitotic divisions, that finally the spores are cut out of a homogeneous plasma by a membrane formed by the fusion of the astral rays of the last generation of nuclei, and that the growing spores are nourished by a food supply which is, at least partly, in the form of glycogen and an unknown substance, the "corpuscules métachromatiques."

The investigations, the results of which are presented in this paper, were undertaken in the hope of extending our knowledge of the ascus to a number of forms hitherto unexamined. A wider knowledge especially of the earlier stages in the development of the ascus and of the immediate details in the forming of the spores seems desirable because of the light that may be shed not only on the question of the affinities of the Ascomycetes among themselves, but also on the larger question of the affinities of the group as a whole.

Probably the most favorable forms that have come under observa-

tion have been *Sordaria fimicola*, *Neotiella albocincta*, and Hydnobolites.

Most of the material was gathered out-of-doors, but the Sordarias and the Podosporas were taken from pure cultures. Several species of Sordaria and Podospora are readily obtainable from horse- or cow-dung brought into the laboratory and kept moist. Pure cultures of any particular species can then be secured by carefully transferring to sterilized dung or other suitable soil, or by catching the spores on a glass slide and sowing them. The objection to dung as a substratum is that it contains sand and small pieces of straw, some of which are sure to adhere to the preserved material and so interfere with cutting. This difficulty was avoided by using green string-beans or dung covered by a thin layer of ground sphagnum moss, on both of which nearly as abundant crops can be grown as on the normal substratum.

Sordaria fimicola was the most serviceable of all the Sordariaceae examined. It is one of the commonest of coprophilous fungi and lends itself to cultivation particularly well. Moreover it matures within a week or ten days after the spores are sown. Further, the perithecial wall is thin and so fixation with such reagents as Flemming's weaker solution is possible, the nuclei are comparatively large, and the ascus is not loaded up with a great deal of diffused reserve matter. Material of *Sordaria fimicola* was preserved at nearly every hour of both the day and the night, but it was found that the various phases in the development of the asci may occur at any time if the proper conditions of temperature prevail. Most of the Podosporas are difficult of fixation. Experiments were made in snipping off the pointed ends of their perithecia with fine scissors, but the results were rather indifferent.

Several species of wood fungi were examined, such as *Massaria vomitoria*, *Massaria platani*, *Propolis faginea*, many of which were gathered in February and March. The most promising of these were the Massarias.

It is worthy of note in this connection, that the apothecium of *Urnula craterium* is well started in the autumn, although the asci do not make their appearance until the following spring. While collecting in November, 1901, in the neighborhood of Bedford, Mass., a number of specimens were found on the underside of oak sticks lying on the ground. They looked like giant Podosporas,

though much lighter in color, and were protected by a hirsute covering. Some had attained a height of 2 cm., and a diameter of 0.6 cm. Several were marked, and placed under further observation. It was found that they matured rather slowly in the spring, for they were not ready to shed their spores until the early part of May.

Several fixing fluids were used, but Flemming's weaker solution was found to be the most satisfactory. Likewise several stains were tried, but Flemming's triple stain of saffranin, gentian violet, and orange G, and Haidenhain's iron-haematoxylin were found to be superior to all others. No hard and fast rule can be laid down for staining. Nearly every species requires a somewhat special treatment. Material for cutting was embedded in paraffin whose melting point was about 57° C. and sections were cut from 3 μ to 5 μ in thickness. In determining the origin of the ascus it was frequently of advantage to crush slices of fresh or preserved material, preferably the latter, and examine in water or potash. Figures 70 to 80 (pl. 11) were drawn from preparations made in this way.

Hydnobolites sp.

Within the bounds of a brief paragraph, Errera ('**82**) recorded the fact that the spores of Tuber are formed simultaneously and not successively, as was formerly believed, that at the moment of their birth they are surrounded by a thin membrane, and that the rest of the protoplasm in the ascus condenses about them to form the greater part of the exospore. Berlese ('**99**) has recently given us a much fuller and more accurate account of the phenomena that occur within the ascus of *Tuber brumale*. Hydnobolites as far as I am aware is the only other hypogeous Ascomycete that has been examined from a modern cytological standpoint. I owe the material to the kindness of Professor Thaxter, who gathered it at Kittery Point, Maine, in September, 1903.

Hydnobolites is admirably adapted to a cytological study. The fruits can be readily cut into thin sections, and each section contains two or three score of asci in all stages of development. The asci are comparatively large, and owing to their spherical shape and the disposition of the spore-forming plasm do not suffer from being cut in any plane. *Tuber dryophyllum* was also examined, but not as fully, for its structural features are on a smaller scale. There is

likewise more objectionable reserve matter in the way, and very few spores are found in an ascus.

The ascus in Hydnobolites springs from the penultimate or terminal cell of the tip of an ascogenous hypha (pl. 11, fig. 78), so that in this respect it may depart from the conventional type. From the first it has the spherical form. In the early stages its protoplasm is granular and irregularly vacuolated throughout, but little attention was paid to the nuclear or other phenomena belonging to this period.

The condition represented in figure 1 (pl. 7) was taken as the starting point for a more careful examination. At this stage there is a very distinct differentiation of the cytoplasm. The greater part of the ascus is occupied by large vacuoles that are filled mainly with watery sap, while there is a small mass of thick protoplasm in the upper part of the sac, against the wall or removed but a short distance from it. Viewed from above, this mass is seen to be of a discoid form, and viewed laterally of a somewhat lenticular shape (pl. 7, figs. 2, 3). A large nucleus lies in the center or along the lower border of the thick protoplasm. It is limited by a clearly definable membrane, possesses a comparatively large nucleolus, and contains a very small amount of chromatin. The last is arranged in the form of fine granules on a filar achromatic network, but the nuclear sap has such an affinity for stains that these features are often almost obscured.

Outside of the nuclear wall at about this time and closely in contact with it there appear small granules that stain a bright red with Flemming's triple, and black with iron-haematoxylin. These granulations have been observed elsewhere and are probably the same as Guilliermond's (:02) "corpuscules métachromatiques." That they are not artifacts as Harper ('99) supposed for similar appearances in *Lachnea scutellata* is very obvious from the fact of their constant occurrence and rather characteristic features at different stages.

Both the nucleus and the dense protoplasmic mass increase in size, the latter frequently passing through a vacuolated condition as represented in figure 2 (pl. 7), while the extranuclear bodies tend to become fewer and larger. This vacuolated condition is succeeded by one (pl. 7, fig. 3) in which the protoplasm is finely granular, though marked by coarse strands that tend to radiate from the nucleus. The latter is still surrounded by the granules or extra-

nuclear bodies, which have apparently increased in number. These granules very soon move away from the nucleus. At first they are scattered throughout the protoplasmic mass (pl. 7, fig. 4) but eventually pass to its periphery (pl. 7, fig. 5). Soon after the first spindle stage they almost entirely disappear.

In the meantime the protoplasm has been undergoing changes. Simultaneously with the dispersion of the extranuclear bodies the protoplasm in the neighborhood of the nucleus becomes hyaline and more finely reticulated. This hyaline protoplasm possesses a radiate structure with the nucleus as a center (pl. 7, fig. 4). These changes are indications that the first nuclear division is near at hand. The transforming process is extended to all of the dense protoplasm, except a coarsely and irregularly reticulated sheath at the periphery, in which the red granules have come to lie. At this stage a centrosome and aster make their appearance, as shown in figure 5 (pl. 7) in a tangential plane that is parallel to the long axis of the spindle that is to follow. The steps leading up to the formation of the spindle were not determined.

The first spindle (pl. 7, fig. 6–13) is generally not as striking as those of the two succeeding generations. It is intranuclear, a condition that lasts until a comparatively late anaphase stage, generally eccentrically placed and terminated by centrosomes from which radiate long rays into the surrounding protoplasm. The rays are very coarse and long and are readily demonstrable with either the triple stain or with the iron-haematoxylin. It is worth noting that they stain differently from the centrosome. They are connected with the centrosome but that they are outgrowths of this body is open to question. Again there is no more evidence that they are retracted to the centrosome at the time of their disappearance, as has been suggested, than that they originated therefrom. Indeed, instead of being resorbed by the centrosome they appear to fade out in the cytoplasm. Moreover, since the latter changes so constantly, as we have seen, it is not difficult to believe that the astral rays are cytoplasmic modifications.

It has further been suggested (Harper, '97) that in *Erysiphe communis* the number of astral rays may be the same as the number of spindle threads and continuous with them. Their existence previous to the appearance of the spindle, their separation from the spindle by centrosomes of even earlier existence than their own, and

both an apparent disparity in their number and that of the spindle threads, and the unlikelihood of the tenuous threads of the spindle fusing through the centrosome with the coarse rays, argue against any such relation between spindle threads and astral rays in Hydnobolites.

Throughout the division of the nucleus the centrosome appears to be considerably augmented by a granular mass. Both the solid and the granular parts are shown in figure 7 (pl. 7). It is likely that the granular portion consists in part of bases of rays lying in other planes than in the one represented in the drawings.

The chromosomes are very small and are four or five in number. The daughter chromosomes separate after cleavage, and on reaching the terminals of the spindle the latter begins to elongate. The result of this is that the nuclear wall is distorted (pl. 7, fig. 10) Finally the nuclear wall disappears, leaving both spindle and nucleolus in the cytoplasm. The spindle threads elongate still farther before their disappearance, the chromosomes in the meantime losing their identity and passing into a tight clue condition (pl. 7, fig. 13). Eventually the astral rays are no longer visible, new walls are formed about the nuclei, each centrosome shows as a small solid mass in a position 90° removed from its position during the division of the mother nucleus, the chromatin again forms a network and each nucleus acquires a nucleolus (pl. 7, fig. 14). The old nucleolus begins to appreciably diminish during the anaphase stages of the division. Its disappearance does not keep step with the development of nucleoli in the daughter nuclei as Gjurasin ('93) thought to be the case in *Peziza vesiculosa*. Another noticeable feature is that the cytoplasm does not now seem to be differentiated as it was during and just prior to the division of the nucleus, and has become rather coarsely reticulated (pl. 7, fig. 14).

After a short period of rest the division of the two daughter nuclei is initiated by the same differentiation in the protoplasm surrounding them that was observed just before the first division. The protoplasm becomes finely reticulated and presents a radially striate appearance (pl. 7, fig. 15). As before, the change in the surrounding protoplasm extends almost to the limits of the dense cytoplasm. The prophase stages are identical with those already observed. It may be noted in this connection that the centrosomes are frequently not demonstrable during the resting periods between the first and

second and between the second and third spindle stages. But they either reappear or else are formed anew in the early prophase stages of mitosis.

The spindles of this generation lie in about the same plane as that in which the first lay, and end to end in a straight line. They are more easily studied than was the first, and though not different from it in the main, yet exhibit certain peculiarities that readily distinguish them. Thus, unlike the first, they occupy a proportionately larger part of the space within the nuclear wall, they are not eccentrically placed, their astral rays are somewhat coarser, and there is a sort of mantle formed about the intranuclear part of each (pl. 7, fig. 16). This mantle, the definite character of which was not determined, adheres to the middle part of the spindle for some time after the nuclear wall goes to pieces. In all probability it is not a mantle of fibers belonging to the spindle, for figure 17 (pl. 8) indicates that there is no connection with the centrosomes. In the anaphase stages the spindle elongates, at first giving to the nuclear wall an oval shape, and after the disappearance of the latter, still continues to extend. The stretching or growth of the fibers, whichever it may be, is the most pronounced in this case of all the three mitoses occurring within the ascus. Finally, the threads of the spindles vanish, and the four daughter nuclei form, and pass into a resting condition, as we saw for the nuclei that preceded them. They change their relative positions, however, so that, while in practically the same plane, they are not in a straight line.

Coming now to the last mitosis it is to be noted that the phenomena up to the anaphase stages are the same as have been described for the second division, and they are as readily observable. The spindles may lie side by side or at an angle to one another, but all are in about the same plane (pl. 8, fig. 19). Interest centers in the events subsequent to the spindle stage, for before the daughter nuclei reach the resting state the young spores are delimited.

We have already seen that one of the features of the anaphase stages of the first two mitoses is an elongation of the spindle fibers. The same is true of the third. The result of this elongation in the last case is to bring the young daughter nuclei against or near the upper part of the plasma membrane that surrounds the dense protoplasm. Thus in looking down on the upper end of the ascus as represented in figure 21 (pl. 8) the centrosomes of five nuclei could

be seen in the same field. Presumably the causes of elongation in this case are the same as in the preceding ones. There is no evidence to show that some of the astral rays become attached to the plasma membrane and then contract, thereby drawing the nucleus up against the membrane.

Another striking anaphase condition is the persistence of the asters after the spindle fibers have disappeared. But this happens with the last generation of daughter nuclei only. They persist until the young spores are formed. This naturally leads us to enquire what part, if any, the astral rays play in spore delimitation.

This question has been investigated by Harper ('97) in a number of forms of which *Erysiphe communis* was apparently best suited for the purpose. His answer in a word was that the astral rays effect the separation of the spore plasma from the surrounding protoplasm by forming the limiting membrane or plasma membrane of the young spore. He states that the eight nuclei of the third generation lie irregularly arranged in the ascus, each with its centrosome and polar rays. A beak then appears on each nucleus on the end of which is the aster. This beak is about equal in length to the diameter of the nucleus and is a small tube sometimes with an expanded top. Into this tube the chromatin network extends. His assumption was that this last fact seemed to prove that there was a union between the chromatin and the centrosome. He regarded the beak as a growth and not as being produced by a pulling out of a side of the nuclear wall. He noted that the beaks were turned toward the ascus wall. As soon as (*before*, according to his figures) the beaks had reached their maximum length, the polar rays experienced a remarkable transformation. The rays began to turn outward, and in moving outward came into contact laterally and fused into a membrane. At this time the membrane formed a funnel or inverted hollow cone the apex of which was at the centrosome. The rays constituting the membrane now began to grow rapidly in length and at the same time to bend down toward the nucleus. The membrane cut through the protoplasm and finally its edges met at a point opposite the centrosome. This completed the process of delimitation and at the same time furnished the spore with a plasma membrane.

Turning now to Hydnobolites we find that the phenomena presented there are apparently quite different. We have already

observed that the eight nuclei of the last generation lie in close proximity to the plasma membrane surrounding the thick protoplasm. As yet their walls are unformed and chromatin and centrosome are indistinguishable. The astral rays are long and prominent, but except for a different position from that represented in figure 19 (pl. 8) they are unchanged. Figure 21 (pl. 8) presents a view of the condition of affairs at this time in polar view, and figure 23 (pl. 8) of perhaps a slightly later stage. From these figures it is evident that the rays have now come to lie in a plane that is nearly parallel to the plasma membrane, that is, in a plane which stands at an angle of 90° to that in which they lay in the equatorial stage of the spindle. Indeed, they are slightly bent down towards the nucleus, a bending that becomes more and more accentuated. The writer, however, dissents from the view (Harper, '97) that the rays are moving down to become the plasma membrane of the spores and that their movements are comparable to the movements of the cilia of zoospores.

Changes in their position might be explained in part by two causes: (1) the outward movement of the nuclei. Thus there is a similar though not as extensive spreading movement of the rays at the end of the first and second mitoses (compare with fig. 12, pl. 7 and fig. 17, pl. 8). (2) Further, if the centrosome be a dynamic center and the rays an expression of cytoplasmic activity controlled by the nucleus, then they would change their position at this time, for now the bulk of the cytoplasm lies centrad of the centrosome, and it is here that aggregation and differentiation of spore plasm is taking place.

It is not impossible that some of the rays pass into the plasma membrane surrounding the dense protoplasm as Harper ('99) has suggested for *Lachnea scutellata*. Cases have been observed in which the rays seem either to stop short at the membrane or to be absorbed by it, but generally they bend away. The same investigator (Harper, '97) corroborates this last observation in the figures accompanying his paper on Erysiphe. Moreover, a reference to figures 11 and 16 (pl. 7) of this paper shows that the development of the rays on the membrane side is less than on the side in which the bulk of the protoplasm lies.

Figures 23, 24, and 25 (pl. 8) represent the next stage, the one in which delimitation of the spore begins. This process is initiated by

the formation of a specialized layer of protoplasm, perhaps of the same nature and formed in the same way as the hyaline zones observed by Harper ('99) in Pilobolus. This layer begins just outside the centrosome and is formed progressively from that point until it encloses the plasma of the future spore. In figure 24 (pl. 8) it is not yet half completed. It is very thin at first, but gradually thickens and since it stains differently from the rest of the protoplasm, is quite clearly definable. That this limiting layer of protoplasm is a membrane, however, is very doubtful, and that it is composed of fused astral rays is contrary to my observations.

That the astral rays do not fuse is readily demonstrable in Hydnobolites. They are very long during the spindle stage, and do not materially alter in length after that. They do change their position, it is true, but the change is one that throws them farther apart instead of bringing them closer together. Fusion is, therefore, a physical impossibility. The only way in which fusion could be effected would be by the flattening of the rays, or by a growth of lateral wings on each. But a polar view at this or any other stage presents no such transformations. The rays are distinct and unchanged, and the limiting layer is of uniform thickness. Indeed, it is quite obvious that whatever the composition of the limiting layer may be, it does not consist of fused astral rays.

While the spore plasma is being separated in this way, it is observable that a change is taking place in its structure. Starting from the nucleus the protoplasm that is to be included within the spore becomes more finely reticulated and eventually more hyaline than that outside. Such a change suggests that there may be a connection between the refining or differentiation of the spore plasm and the formation of the limiting layer.

About the time the limiting layer is being constructed, the nuclear wall is formed. When the completed nucleus is first demonstrable, it presents the appearance of a short cylindrical vesicle. It then elongates, growing towards the center of the spore mass, or to one side or the other at any angle (pl. 8, figs. 26, 27). Meanwhile it retains its cylindrical shape. Eventually it grows beyond the middle of the spore, so that its length may be equal to three quarters the diameter of the spore. Likewise it becomes bottle- or flask-shaped, the neck becoming quite tenuous (pl. 8, figs. 29, 32). But this "beaked" shape is not evident until long after the spore is delimited.

The early elongation of the nucleus is undoubtedly accompanied by growth. It may be that, in addition to this, there is a tension brought to bear on the nucleus, a tension that may have its origin outside of the spore altogether (pl. 8, figs. 29, 32). That the bottle- or beak-shape is the result of a pull on the nucleus is rather more obvious, for there is an elongation without an appreciable increase in volume. Nevertheless this bottle-shape might be brought about through other changes, such for instance as the transfer of the chromatin and nuclear sap to the distal end of the nucleus, and the consequent shrinking of the proximal end. If there be a pull, however, it is not strong enough to pull the centrosome away from the delimiting layer of protoplasm. Indeed the centrosome does not sever its connection with the periphery of the spore mass until after the plasma membrane of the spore is constructed.

The formation of the plasma membrane of the spore is subsequent to that of the limiting layer of protoplasm already noted, although it may begin before the latter is completed. Figures 26 to 30 (pl. 8) represent this membrane in various stages of growth. In figure 26 (pl. 8), only one of the poles of the spore is covered while in figure 29 (pl. 8), the membrane is all but finished. Figure 33 (pl. 8) is a polar view of about the stage represented in figure 29 (pl. 8).

Concurrently with the formation of the plasma membrane of the spore, another is formed in contact with it. This second membrane lines the cavity in which the young spore lies. Both occupy the site of the limiting layer of protoplasm, and along this their line of development follows. The development of these membranes is progressive, beginning at a point contiguous to the centrosome and continuing until the opposite pole of the spore is reached. To just what extent the substance of the limiting layer enters into the composition of these membranes, it is rather difficult to say. Nor is it clear that their formation is preceded by a change in the limiting layer. It is certain, however, that the membranes are separable and a cleavage between them can be observed under favorable circumstances up to the limits of their construction. Observation is rendered especially easy in case there has been a slight shrinkage or inequality in fixation (pl. 8, figs. 29, 33).

In regard to the relation between the plasma membranes and their precursor, the limiting layer, it is highly probable that the development of the former is preceded by a cleavage in the latter. They

are certainly not identical. The limiting layer is thin at first, becomes gradually thicker, but is never as clearly definable at its edges as are the membranes that follow. It consists of a substance that possesses a different staining capacity from the surrounding cytoplasm and while the cytoplasm is more or less coarsely reticulated or foamy in structure, it on the other hand is apparently structureless or very finely granular. But it is never separable from the protoplasm on either side. The cases of separation figured by Harper are evidently separations between the plasma membranes. Now it is not improbable that with the increased growth and differentiation of the limiting layer, and a pull on the part of the nucleus, that the cleavage is first affected in the part adjacent to the centrosome. It is here that the membranes first begin to form, and it is this part that would be directly affected by a nuclear tension. A continuance of cleavage would be favored by the formation of membranes on the surfaces already separated and much more by the differentiation of the spore plasma which by this time at least is in course of progress.

The membrane covering the epiplasm undergoes no further change except in extent and shape according as the spore changes in this respect. It continues to line the epiplasm until the spores are developed, and is the last of the epiplasmic substance to disappear. The membrane covering the spore plasm likewise continues for a time without other changes than in extent and shape to meet the demands of the growing spore, and the various pressures brought to bear upon it. When the spores are nearly matured, however, the exospore begins to be laid down, and with this process perhaps both plasma membranes are intimately connected.

Subsequent to the formation of the plasma membranes one of the chief matters of interest until the spores are mature, consists in the resumption of a spherical form by the nucleus. This change takes place very soon after the completion of the membrane, though the nucleus may first elongate still farther as shown in figure 32 (pl. 8). The neck of the flask-like nucleus contracts, and the result is to thicken it and draw the centrosome away from the periphery towards the center (pl. 8, fig. 34). In one respect Hydnobolites differs from the forms already described, for as yet the astral rays have not vanished. Indeed, they are demonstrable until the beak is fully withdrawn (pl. 8, fig. 35), although at that time they begin

to fade out. As soon as the centrosome begins its retreat, the rays begin to spread out in all directions.

Whether these rays are precisely the ones that were found extending out from the centrosomes in the spindle stage cannot be affirmed with absolute certainty. But there seems no reason to think that they are any other, nor that they do not include practically all of those rays. This being the case, there is corroborative evidence that the layer of protoplasm that first delimited the spore does not consist of fused astral rays.

Neotiella albocincta (B. & C.) Sacc.

Harper's ('99) description of spore formation in *Lachnea scutellata* is the most detailed account we have of the cytology of the ascus in the Discomycetes. He begins with the uninucleate condition just prior to the first mitosis, and his observations on this stage correspond with those already given by De Bary and Gjurasin. Passing on to the last mitosis, he notes that the daughter nuclei acquire walls before "spore-cutting" is begun. They lie close to the wall of the ascus with their asters directed outward. The base of the nucleus next grows towards the center of the ascus and in this way a beak is formed. The formation of the spores takes place in the manner previously indicated for Erysiphe, so that in Lachnea, too, the spore plasm is "cut" out by a plasma membrane consisting of fused astral rays. He found that there was no aggregation of protoplasm at the time the spores were delimited. He had previously described (Harper, '97) an aggregation in *Peziza vesiculosa* as far back as the four-nucleated and four-spindle stages, but concludes from a re-examination that his previous observations were wrong.

Neotiella albocincta has proved valuable in a study of this kind because of the fact that its nuclei are unusually large, and its protoplasm is free from undesirable substances. Extranuclear bodies are of rare occurrence. The stages in the early development of the ascus, too, are readily followed, but this phase of the subject will receive consideration later on. The material was collected in small quantity near Toronto, Ontario, in the latter part of October, 1902.

Beginning with the mature uninucleated condition (pl. 9, fig. 40) it is noticeable that, unlike most forms, the ascus has not yet attained its maximum size. There is the same differentiation of

protoplasm that Harper noted in Lachnea though the dense portion is not sharply bounded off by concave surfaces, but streams out irregularly into the foamy protoplasm above and below. The nucleus occupies the middle of the dense cytoplasm, though this position is by no means a fixed one. It is perhaps the largest nucleus so far recorded for the Ascomycetes. It possesses a large nucleolus, and well defined and abundant chromatic threads. At this stage, with Flemming's triple, the nucleolus stains a bright red, and the chromatin blue.

The first spindle stage is characterized by a number of interesting features. Throughout karyokinesis the reaction of the chromatin towards the triple stain is changed, for now it dyes a bright red. The spindle is especially clear (pl. 9, fig. 41). It is comparatively broad at the middle and tapers at both ends to terminate in very small centrosomes. From these centrosomes there stream out fine rays which are exceedingly difficult of demonstration. Indeed, in figure 42 (pl. 9) in which the centrosomes lie in the plasma membrane of the ascus, the astral rays appear to be lacking. The long axis of the spindle may lie in any plane from the vertical to the transverse. The nuclear wall degenerates early, and may be gone before the equatorial-plate stage is reached. The nucleolus suffers a very apparent reduction in size during the construction of the spindle. The chromosomes are large, six or seven in number (judging from the number of daughter chromosomes, figure 41), and are horseshoe-shaped. At first the arms of the horseshoe are thick, but as they are pulled back towards the poles of the spindle, they become much more attenuated (pl. 9, fig. 43). During the anaphase stage the centrosomes are no longer to be seen. If present they are obscured by the masses of chromatin. The daughter nuclei are prominent and often contain two nucleoli. But in these as in all resting nuclei belonging to this plant, no centrosomes could be detected.

The details of karyokinesis in *Peziza rutilans*, lately described by Guilliermond (: 03a) bear a close resemblance to those I have just described for Neotiella. There are protochromosomes in both, and the chromosomes are U-shaped, especially in the anaphase stage, but twelve in number in *Peziza rutilans*. Guilliermond states that the nuclear wall disappears in the prophase stage, and that neither asters nor centrosomes exist. Because of the absence of astral rays,

an account of the method of spore formation would have been highly desirable.

Though on a smaller scale, the divisions of the second (pl. 9, fig. 44) and the third (pl. 10, fig. 49) sets of nuclei are practically repetitions of the first. It is noticeable with them, too, that the spindles may lie in any plane. The chromosomes, however, in both and especially in the third set are much more slender and more closely massed together. During the last mitosis the protoplasm begins to aggregate about the nuclei.

It is towards the end of this stage that spore delimitation begins. The daughter chromosomes have massed together at the poles of the spindle, and the latter has elongated bringing the eight immature nuclei near the walls of the ascus (pl. 9, fig. 45). The spindles may lie in any plane. Extending out from the poles in some cases can be detected a curved hyaline line, the beginning of the limiting layer similar to that described for Hydnobolites. Walls now soon form about the nuclei which immediately after grow in towards the center of the ascus, in this way acquiring a beaked shape (pl. 9, fig. 46). Their chromatin has passed into a reticular condition, though still massed in the beaked end of the nucleus. Already a nucleolus has made its appearance. The limiting layer of protoplasm advances progressively until completed at the opposite pole, and is considerably thicker than when first seen. At this time the spore plasm is distinctly differentiated.

The succeeding stage (pl. 9, fig. 47) is characterized mainly by an elongation of the beaks, by some growth of both nucleus and spore plasmas, and a continued differentiation of the latter. The plasma membranes then begin to form.

These plasma membranes as in the case of Hydnobolites are two in number: one covers the spore plasma and the other lines the epiplasm in which the spore lies. They are formed, too, on the site of the limiting layer. Their organization begins adjacent to the centrosomes, and, associated with the process of cleavage, continues along the limiting layer until the opposite end of the spore is reached. It is at this stage that surface tensions indicative of a plasma membrane are first apparent. In figure 48 (pl. 9) the membranes are but half formed, but in figure 50 (pl. 10) they are complete. The upper spore in figure 50 (pl. 10) shows an interesting phenomenon that occurs occasionally, namely, the possession of two beaked nuclei.

The spore plasm has in the meantime become very finely reticulated, and the epiplasm is very coarse. Likewise the spore has assumed a regular oblong shape which is retained. The nuclear beak retracts, but in so doing the bulb of the flask is drawn up some distance towards the attached end (pl. 10, fig. 51). The centrosome has disappeared. A period of growth now ensues for both the spore and its nucleus (pl. 10, fig. 52). After the spore has reached about its maximum size the exospore is developed, and when this is finished the epiplasm is practically exhausted.

Sordaria fimicola (Rob.) Ces. & DeNot.

We owe our knowledge of the cytology of the ascus in the Perisporiales to the investigations of Professor Harper ('97) on *Erysiphe communis*. Outside of this order the only member of the Pyrenomycetes to receive attention has been *Cordyceps ophioglossoides*, and this at the hands of Lewton-Brain (:01). Unfortunately Lewton-Brain attempted a cytological investigation on material that was not suitably fixed. He even failed to see the nuclei, evidently mistaking the nucleoli for them. He states that no formation of spores takes place until all nuclear divisions are completed. The nuclei then arrange themselves in eight rows, and the delimitation of these rows and the separation of their nuclei by septa follow. It is in this way that the septate spores originate.

The writer has examined the same form preserved in Flemming's weaker, and finds that the nuclei possess membranes, chromatin, and nucleoli. The only respect in which they deviate from the type is in the possession by the fusion nucleus of small cones. These cones are extranuclear and lie outside the nuclear wall at opposite poles. The nuclear divisions are likewise typical. All the stages in spore formation were not followed, but there is reason to believe that the young spores are uninucleate, and that the multinucleate condition arises by nuclear divisions within the spore.

The Pyrenomycetes are, in general, for various reasons, difficult to manipulate. Of the forms studied none has proved more favorable for examination than *Sordaria fimicola*. The methods employed in growing material have already been described.

The ascus most frequently springs from a curved terminal cell of an ascogenous hypha, much as in the case of *Genea hispidula* (pl.

11, fig. 76). Sometimes, however, the tip is segmented off in the conventional manner, and then the ascus grows out from the penultimate cell. Not all the nuclear phenomena have yet been elucidated, but such as have been, present no new features.

Early in the development of the ascus, extranuclear bodies make their appearance about the "fusion" nucleus. They are in the form of granules or flat masses which lie close against the nuclear wall. They are not scattered about the protoplasm or away from the nucleus as was found to be the case in certain stages in the development of the asci of Hydnobolites. Figures 60 and 61 (pl. 10) represent similar bodies in species of Podospora, in which, however, they are larger than in *Sordaria fimicola*. The number of these bodies is at first small, perhaps not more than one or two, but later on there may be a dozen of them around a single nucleus. Just before the first spindle is formed they disappear and do not again occur. They are probably homologous with Guilliermond's "corpuscules métachromatiques."

There is the same differentiation of protoplasmic contents that we have already observed in *Neotiella albocincta*, and the first nucleus lies within the thick protoplasm. This nucleus, however, does not contain nearly as much chromatin as the corresponding one in Neotiella, though the achromatic network is well developed. The centrosomes throughout are flattened and disc-shaped in the resting nuclei, but smaller in those that are dividing.

The first spindle is prominent, though very slender, is intranuclear, and possesses long, fine astral rays which are demonstrated with some difficulty. Its long axis lies in the long axis of the ascus or very nearly so. In connection with the second set of spindles there are no new features, and the only point to be mentioned is the same extreme lengthening in the anaphase stages that has been noted elsewhere. The resting nuclei at the end of this division attain a fairly large size, and their centrosomes are particularly clear (pl. 10, fig. 53).

During the four-nucleated stage, a differentiation of the thick protoplasm may begin, though the heaping up of the protoplasm about the nuclei becomes much more obvious during the spindle stage that follows. From figure 54 (pl. 10) it will be seen that the protoplasm in the immediate neighborhood of the nuclei is finely reticulated, while farther away vacuoles or coarser reticulations are

evident. This differentiation of spore plasm becomes more and more pronounced from this time on. During the closing stages of the last mitosis the spindle grows out through the ends of the nuclear wall, leaving behind the nucleolus and what are perhaps collapsed remains of the wall. The chromatin is massed against the centrosomes which have come into very close relation with the plasma membrane of the ascus. Just what has caused this elongation and just what the nature of the connection is between centrosomes and plasma membrane are difficult questions to answer. Fine astral rays stream out from the ends of the spindles of the last set and it may be possible that some of these do pass into the plasma membrane and then contract, the contraction resulting in the elongation of the spindle and the drawing of the centrosomes to the wall. This would explain the depressions in the plasma membrane adjacent to the centrosomes and their connection with the former (pl. 10, fig. 55).

The explanation offered by Harper ('99) of similar depressions in Lachnea was that they were caused by the astral rays pulling out of the membrane preparatory to "cutting" out the spore mass. Obviously such an explanation assumes an exercise of considerable force by the rays, for unless the pull be straight in line with the spindle the rays are at a mechanical disadvantage. Moreover, unless the pull be in line with the spindle (which assumes that the middle rays must be very short, which is contrary to fact) the center of depression would not be adjacent to the centrosomes.

The limiting layer of protoplasm that separates the spore plasm of a future spore from the epiplasm begins to form during the final anaphase stages of the fourth spindle (pl. 10, fig. 55). It extends out from the centrosome following for a way closely along the plasma membrane that lines the wall of the ascus. The layer is progressively formed until completed at the pole opposite the centrosome. Primarily very thin, it eventually thickens and becomes more conspicuous. Meanwhile the walls have formed about the daughter nuclei and this is immediately followed by a growth of the nuclei resulting in the flask shape or beaked form (pl. 10, fig. 56).

As soon as the spore masses are delimited, the plasma membranes are developed. They are formed in the plane of the limiting layer, and the phenomena of their production are the same as described for Hydnobolites and Neotiella.

The young spores when first delimited, are much compressed in shape, but they soon acquire the oblong shape indicated in figure 57 (pl. 10). In figure 57 the plasma membrane is completed and the nuclear beak has reached its maximum length. This beak has become more tenuous than formerly and the chromatin has been distributed.

The centrosome soon breaks from the plasma membrane and the beak is retracted. This retraction is preceded by a contraction which results in its thickening and in drawing the venter of the flask nearer the end of the spore.

This re-formation of the nucleus is directly followed by its division. The division is karyokinetic. The spindle is intranuclear and there are asters. In the anaphase stage it elongates beyond the limits of the nuclear wall which soon after disappears (pl. 10, fig. 58). When completed, the daughter nuclei resemble the mother nucleus in all respects except in size (pl. 10, fig. 59). The exospore has not yet been laid down, though its appearance is now not long delayed.

Septate and Multinucleate Spores.

Multinucleate spores are generally septate. *Sordaria fimicola*, as we have seen, affords an example of a departure from this rule. The spores of *Discina venosa* are several-nucleated and yet not septate, while those of a Thielavia found by Professor Thaxter at New Haven, growing parasitically on Isaria, regularly contain four nuclei. When first formed, all possess but a single nucleus, the increased number arising by subsequent divisions proved in *Sordaria fimicola* to be karyokinetic.

Septate spores are likewise uninucleate at their origin. This has been shown to be the case in various forms examined by the writer, such as *Massaria vomitoria*, *Massaria platani*, *Geoglossum ophioglossoides* and *Geoglossum hirsutum*, *Leptoglossum* sp., and *Leptoglossum luteum*. The spores are nearly spherical when delimited, but after the development of the plasma membrane they begin to elongate. In course of this growth in length the nuclei divide and septa are usually formed after each division, thereby separating the daughter nuclei from one another. The nuclei seem to be intimately connected with the construction of septa, for septa are formed in

their immediate neighborhood only. In *Cordyceps ophioglossoides* young spores were observed that contained but a single nucleus and in all probability the subsequent steps in development do not materially differ from those occurring in a Massaria or a Geoglossum.

The spores of several of the Podosporas present a curious case of the formation of a septum without a previous division of the nucleus. This practically amounts to cutting off from the spore a part that persists as an appendage or tail and maturing the part only in which the nucleus lies. Such tailed spores occur in *Podospora setosa*, *P. anserina*, *P. fimiseda*, *P. curvula*, etc. *Podospora curvula* illustrates very well the steps in the development of such spores. The preliminary stages are similar to those that take place in *Sordaria fimicola*. The spore plasm is also delimited as in Sordaria, though early aggregation of the protoplasm is not so readily observable. The young spores are at first compressed (compare with figure 56, plate 10) but rapidly change in form so that the nucleus appears to be attached to the end and not to the side of the membrane. The shape of the spore at this time is cylindrical or rod-like. This is followed by an enlargement of the upper end in which the nucleus is to be found. It next moves down to the entrance of the small end (pl. 10, fig. 62), for it has grown too large to go any farther. This step is preparatory to the cutting off of the tail.

A septum is evidently formed through the activity of the nucleus. That the latter is more active than usual is shown by the fact that the cytoplasm in its immediate neighborhood gives a staining reaction with Flemming's triple different from that in the rest of the cytoplasm (pl. 10, fig. 62). In any case a wall is laid down at this time and just below the position occupied by the nucleus. The wall having been formed the nucleus passes once more to the center of the upper part (pl. 10, fig. 63). The tail develops no further, and gradually loses its protoplasm. Whether or not the septum partakes of the character of a plasma membrane may not be decided with certainty. But the exospore is developed in connection with this part as with the rest of the wall surrounding the nucleated portion. That it is developed but very slightly on the tail, which grows but little more, is proof that the nucleus to some extent at least controls the laying down of the exospore.

Origin of the Ascus.

According to the researches of Dangeard ('94) and Harper (:00) the ascus originates from the cell next to the terminal one. After an examination of thirty-six species from which it is possible to determine this matter, it has been found that such is invariably the case in but eleven of them.

Maire (:03) and Guilliermond (:03a) have recently reported deviations from the conventional type in *Aleuria cerea* (?), and *Galactinia succosa*. In the former the ascus arises from a penultimate cell in which there are two nuclei, but the terminal cell likewise contains two nuclei. In *Galactinia succosa* the end of the hypha is not hooked, the last two or three cells enclose two nuclei each, and the ascus springs from the terminal cell.

The most frequent deviation from what has been taken as the conventional type is that represented in figure 74 (pl. 11) for *Genea hispidula*. In this case the ascus grows out from a curved terminal cell. It is quite probable that the only departure from the type is the lack of the cross wall that cuts off a uninucleated cell at the tip of the hypha. Other forms that resemble *Genea hispidula* in this respect are *Podospora anserina*, *P. setosa*, *Sordaria fimicola*, *S. humana*, *Geoglossum ophioglossoides*, *G. hirsutum*, *Geoglossum* sp., *Verpa conica*, *Gyromitra sphaerospora*, *Leptoglossum luteum*, *Leptoglossum* sp., *Mitrula phalloides*, *Leotia lubrica*, *L. chlorocephala*, *Phyllachora graminis*, and *Discina venosa*. In some of these the missing partition is found to be present exceptionally.

Of all the forms examined, *Verpa bohemica* exhibits the greatest variability. It has been hitherto stated that asci spring from the penultimate cell and that the terminal one develops no further. Both of these statements may be incorrect as far as *Verpa bohemica* is concerned. Thus, asci sometimes occur as represented in figure 79 (pl. 11) as outgrowths of a terminal cell. In the case figured there is a second outgrowth which is perhaps of later origin than the ascus. Again, the ascus may be found to grow out from the second, third, or even fourth cell from the end (pl. 11, figs. 71, 72, 75, 77). It remains to be determined yet whether the terminal cell underwent divisions after the budding out of the ascus, or whether the ascus may grow out of the third or fourth cell as it sometimes does from either the first or the second. In *Anixia*

spadicea (pl. 11, fig 80) and *Urnula craterium*, it looks as if the ascus might spring from any cell whatever. The Anixia was obtained from a culture given me by Professor Thaxter.

A number of forms were found to follow very closely the type described by Harper and Dangeard. These were *Peziza* sp., *Neotiella albocincta*, *Morchella esculenta*, *M. conica*, *Helvella atra*, *H. lacunosa*, *H. elastica*, *Acetabula* sp., *Pseudoplectania* sp. In Hydnobolites and Elaphomyces the ascus usually grows out from the second cell, though occasionally the end septum is lacking.

The cytological phenomena in connection with the origin of the ascus are quite readily worked out in the Helvellas, Neotiella, and Peziza. Neotiella was found an especially favorable form because of the size of its nuclei and the absence of extranuclear bodies.

The earliest stage of all, however, was observed in *Peziza* sp., in which the hooked end of an ascogenous hypha was found with a nucleus in each arm of the hook. These nuclei were also observed in a condition of mitosis (pl. 11, fig. 64). Of the four daughter nuclei the proximal one is soon shut off by a septum (pl. 11, fig. 65). This was the first stage found in Neotiella. A second septum was then formed cutting off the distal nucleus (pl. 11, fig. 66) and leaving the two middle nuclei in a penultimate cell. It is from this cell that the ascus grows out. The two nuclei fuse either before or after they enter the young ascus (pl. 11, fig. 69). In *Urnula craterium* the fusion is always effected in the ascus and not until it has attained a considerable length.

This fusion apparently consists in the disappearance of the parts of the nuclear walls in contact, and the flowing together of the chromatin and the nucleoli. The "fusion" nucleus rapidly rounds up unless the fusion has taken place in the ascus. When the "fusion" nucleus lies in the ascus it is always elongated because its diameter is greater than that of the ascus (pl. 11, fig. 68).

The nuclei of the first and third cells usually disappear very soon after the ascus has begun its growth, and the same is true of the protoplasm in the cells in which they lie. It is too much to say, however, that such is the constant fate of both, and especially of the proximal one. The origin of the four nuclei found in the hook cannot be stated with absolute certainty. There is reason to believe that they may be products of a single nucleus in some cases. Thus it is a matter of observation that an ascus-bearing hook may be

developed from a cell removed but one from an ascus and this cell may contain a single nucleus (pl. 11, fig. 66).

General Considerations.

The problem of the origin of the Ascomycetes must be attacked along two main lines, namely, a determination of the homologies of the ascus, and an appreciation of the phenomena associated with the sexuality of the group. These, it is to be pointed out, are separate questions, although it is also true that a final solution can be effected only after a consideration of both. The present research has only to do with the question of the homologies of the ascus.

Asci are now generally regarded as asexually produced spore sacs on hyphae which are outgrowths of a fertilized organ. Most botanists agree on the view that the Ascomycetes have not lost the sexual method of reproduction, and that in some of them at least there are functional sexual organs. What was a conviction of De Bary's has been proved by the researches of Harper (:00) and Thaxter ('96) to be a reality. The sexual organs are borne on a mycelium that follows the germination of the ascospores, and from the fertilized oogonia or ascogonia there grow out the ascogenous hyphae, and it is from the cells of the latter that the asci arise. These observations may, I think, be assumed without further remark to be correct. The next question that arises, and on which there is not such unanimity, is in regard to the ancestral homologue of the ascus.

On this subject four suggestions have been offered: (1) that the ascus is a descendant of the sporangium of the higher Mucorineae through the Hemiasci, a theory that was propounded and stoutly maintained by Brefeld; (2) that the ascus is a derivative of the sporangium (or zoosporangium) of the Oomycetes; (3) that it is a new feature without a homologue outside the Ascomycetes; and (4) that it is a modification of the carpospore of the Florideae.

The phenomena of the early stages in the development of the asci unfortunately do not throw much light on this matter, unless, indeed, to confirm the view that they are coenogenetic organs. The fusion of nuclei within the young asci, the phenomenon to which I especially refer, is a puzzling feature, one that is as yet

unknown to occur in the history of any Phycomycetous sporangium or Floridean carpospore. Dangeard ('94), who first made its discovery, considers it a sexual act, but this conclusion scarcely seems plausible because the following facts appear to have been satisfactorily demonstrated in several instances: (1) there has already been a fusion of sexual elements in the ontogeny of the individual, (2) the fusing nuclei in the ascus are division products of nuclei belonging to the same cell, and perhaps in some cases even daughters of sister nuclei, and (3) they are vegetatively active before fusion, as is the single nucleus after fusion. The phenomenon is probably vegetative rather than sexual, but its nature and significance will not be fully understood until further research reveals whether or not it is an acquired feature.

Fortunately more exact comparisons can be instituted in dealing with the later events in the life of the ascus. Hence the homologies of the ascus will at present be dealt with mainly from the standpoint of the phenomena of spore formation, and in the light of these phenomena each of the foregoing suggestions will be briefly considered in turn.

Brefeld was the champion of the first suggestion for he regarded the sporangium of Mortierella or a closely related form as the ancestor of the ascus, but it is doubtful if his position is tenable on cytological grounds, though the cytology of this particular genus has not been described, yet owing to the careful work of Harper ('99) and recently of Swingle (:03) we know quite fully the cytological details of spore production in such closely allied genera as Pilobolus, Rhizopus, Sporodinia, and Phycomyces. In all of these forms, spore formation is initiated by a segregation of the spore plasm into a cell, the sporangium. The next step consists of a progressive cleavage of the spore plasm into irregular multinucleate masses, the cleavage beginning at the surface of a plasma or a vacuolar membrane. There is no residue of epiplasm. These masses may round themselves up into spores at once as in Sporodinia, or they may undergo further cleavage. In the latter case the later cleavage furrows do not curve, intersect, and branch in the same irregular way as do the earlier, but they seem to bear a more definite relation to the nuclei and in Pilobolus are first marked out by a development of hyaline zones. Harper is therefore apparently justified in concluding that since the method of sporogenesis in these sporangia dif-

fers in so many important respects from what has been described for the asci, it is difficult to see how the asci could possibly have been derived from them.

Nor has a study of Brefeld's so called intermediate forms revealed a connecting link between the Zygomycetes and the Ascomycetes. A number of the Hemiasci have been examined from this point of view by Popta, Holtermann, and Juel, and though some points have not been cleared up, yet the conclusion is fairly safe that their sporangia do not occupy the intermediate position that has been claimed for them.

Holtermann ('98) has described the method of spore formation in a Hemiascous form called by him *Oscarbrefeldia pellucida*. He says of this that in the young ascus the granular protoplasm lines the wall, while the center is occupied by hyaline cell sap. Soon a plate of protoplasm forms across the ascus dividing the central cavity into two parts. In a similar manner the cavity is finally divided by a succession of protoplasmic plates into as many as thirty-two hollow spaces, the interior of the future spores. These small cavities then round themselves up, a cuticularized layer is formed about them, and the spores are completed. There is a residue of epiplasm. He confesses that "Ich habe mich hier auf eine einzige Art [the culture method] beschränkt"; but gratuitously adds "aber nach den vorläufigen Untersuchungen hege ich keinen Zweifel, dass die Bildung and Differenzierung der Sporen bei den anderen Ascomyceten nicht wesentlich abweicht von der des oben beschriebenen Falles." The unfortunate part of his investigations was his failure to recognize the existence of nuclei in fungi. "Denn es würde sich dann sofort herausgestellt haben, dass das, was bis jetzt für Zellkern bei den Pilzen gehalten wurde, theils Vacuolen, theils Protoplasmaansammlungen, theils auch Protoplasmakörnchen sind."

Observations on three of the Hemiasci, *Ascoidea rubescens*, *Protomyces macrosporus*, and *P. bellidis* have been recorded by Popta ('99). In *Ascoidea rubescens* the spores are formed by the protoplasm becoming more densely aggregated about the nuclei, followed by a development in an undiscovered manner of limiting walls. There is an epiplasm. In each of the other forms the protoplasm arranges itself in a parietal layer leaving a vacuole in the center. The division planes in *P. bellidis* are radially arranged, suggestive

of the method of spore delimitation in Saprolegnia. While there is but one layer of spores in this species, there are three in *P. macrosporus*, and in both there is no epiplasm. It is to be regretted that Popta examined living material only.

And finally Juel (:02) has described the process of sporogenesis in *Taphridium algeriense*. According to his description the protoplasm is differentiated into a denser portion that lies at the periphery and in which the nuclei are arranged in a single layer, and a vacuolated portion that fills the rest of the sporangium. Uninucleated spores are now formed from the dense protoplasm, but Juel could not determine how delimitation was effected.

The details of spore formation in the Hemiasci given us by these investigators are apparently too meager to enable us to come to a final conclusion, but it seems quite unlikely from such as are known that the "sporangia" of the Hemiasci are intermediate between Mortierella-like sporangia and asci. It is evident that the latter are not only not directly connected, but also that they are not linked to one another by means of such forms as Protomyces and Taphridium. Nevertheless I cannot agree with Harper (`99) that "the total dissimilarity of the process of cleavage in the sporangia described [Pilobolus and Sporodinia] and the ascus, makes it necessary to look for the ancestors of the Ascomycetes elsewhere than in the lower Fungi," for it is to be noted that the forms he investigated were of a highly specialized type. If the ancestral homologue of the ascus is a sporangium at all, it is more likely to be found among more primitive sporangia than Harper described. This brings us to the second suggestion, namely, that the ascus is a derivative of an Oomycetous zoosporangium.

Spore formation in the lower Phycomycetes has received some attention at the hands of Hartog (`88), Rothert, Humphrey (`93) and more recently Davis (:03). In their descriptions of the cytology of the zoosporangia of the Saprolegniaceae they agree on the general features. The central part of the sporangium becomes vacuolar, the vacuoles flow together and form a large cavity filled with sap. The protoplasm thus occupies a peripheral position. Radial clefts then start from the vacuolar membrane cutting the cylinder of protoplasm into the spore origins, which contain a single nucleus each, and which without further division are transformed into zoospores.

Leaving aside for a moment their relative ontogenetic values it

is to be enquired if the sporogenesis of such a sporangium could not be homologized with that of the ascus, or in other words if the ascus might not find its ancestor in a Saprolegniineous zoosporangium. If so, then we may with more reason look for the ancestors of the Ascomycetes among the lower fungi. There are, of course, marked differences in the sporogenesis of zoosporangium and ascus, but that is to be expected, — they exist between even the sporangia of Saprolegnia and Mucor, which are with little doubt rather closely allied, — for if the Ascomycetes be connected phylogenetically with the Saprolegniineae they are removed at a great distance as is shown by the characters of their vegetative bodies and organs of reproduction, differences which, it is needless to say, have arisen through the acquirement of very different habits. But these differences may not be found to be irreconcilable with a theory that connects the two phylogenetically. For instance, a most striking difference is the fact that the sporangium is multinucleate from the beginning while the ascus starts in a binucleate condition; but this might be accounted for without difficulty if it is granted that a septate thallus, the cells of which are one- to few-nucleated, may arise from a coenocytic thallus. Likewise it will be found that other differences are not so formidable as they appear at first sight, and that taken with the similarities they may not be such as to debar connecting the one with the other.

In the preparations leading to the formation of the spores there are two phenomena which in general are the same in both the zoosporangium and the ascus, and which may not be without significance: (1) the differentiation of the protoplasm into dense and vacuolated portions, (2) the migration of the nuclei to a peripheral position in the dense protoplasm. In regard to the differentiation of the protoplasm, it is worthy of note in this connection that in the ascus, vacuoles frequently occur in a more or less central position directly following the last nuclear division. Harper ('99) noted these vacuoles in Lachnea, but ascribed them to the escape of nuclear sap in the anaphase stages of the last mitosis. This explanation does not seem adequate, however, to account in many cases for the size, number, and position of all of them, even if it be granted that the escape of nuclear sap might be responsible for some.

From this point on, the processes are apparently much more

unlike, and the following differences have been insisted upon by certain botanists as being incompatible with any view that homologizes ascus and sporangium: (1) that in the sporangium the nuclei are perhaps not at all functional in the delimitation of the spores, while in the ascus they are the direct agents; (2) that the process of spore delimitation in the first case is an act of cleavage (previously or not at all marked out by hyaline areas), while in the latter it is a literal cutting out of the spore plasm by a plasmatic membrane; (3) that the plasma membranes of the sporangiospores are extensions of previously existing plasma membranes, while those of the ascospores are composed of fused astral rays; and (4) that there is an epiplasm in the ascus, but none in the sporangium, and that this is because of two fundamentally unlike methods of spore delimitation.

There is, I am inclined to think, some question as to the validity of the first of these contentions in regard to the sporangium. Thus, as far as Saprolegnia is concerned, nuclear control in spore formation seems almost a certainty, for the spores that are formed are constantly uninucleated and never enucleated, and the nuclei are practically the centers of the cleavage areas. And even in the Zygomycetes it is difficult to conceive of control being entirely transferred to the cytoplasm, for, in the forms that have been investigated, it is notable that nuclei are never absent from the protoplasmic masses resulting from the cleavages. Incidentally it may be observed that if spore formation in the Zygomycetes is due to the activity of the cytoplasm, but in the Oomycetes to the activity of the nuclei, then it would appear that the value of the cytology of spore formation as a factor in deciding phylogenetic problems between fairly widely separated groups is rather questionable.

Nuclear control in the Ascomycetes is undoubted. Indeed, certain phenomena demand comment. Thus even the shape of the nuclei changes throughout the process, a feature that seems unique. Timberlake (:02), however, has observed somewhat similarly shaped nuclei in some cases in Hydrodictyon, and his observation was that the pointed end lay near the newly formed plasma membranes and that the occurrence was only noted when cleavage followed closely on nuclear division. In explaining this phenomenon it must be borne in mind that spore-forming in the Ascomycetes is precocious, that the spores are being delimited and the spore plasma differenti-

ated concurrently with the growth of the nuclei, and frequently with their reconstruction, and that these changes are taking place in the same direction as that in which the nuclei are elongated. The question of the part played by the astral rays, however, is a more serious one. That they certainly do not form the limiting membrane but are enclosed within the spore is maintained by the observations recorded in this paper. But this does not entirely dispose of them, for it may be suggested that they serve to direct the delimitation of the spore, especially the laying down of the limiting layer of protoplasm (though not forming a part of it) which marks out the cleavages to follow or as Berlese ('99) thought, of the plasma membrane itself. If they are visible expressions of nuclear forces, that is possible, though rather indicative of cytoplasmic activity under nuclear control in the region in which they lie than of an activity or force within themselves. The variability of their appearance leaves it open to question that they are at all essential to the process or take any share in it. In Hydnobolites they are pronounced; in *Neotiella albocincta* they are not abundant, and are very tenuous and difficult of demonstration, and Guilliermond (:03a) claims that in *Peziza rutilans* they are entirely absent. Indeed, the function of astral rays in the ascus remains the same debated question as in other places in which they occur.

As to the second and third objections, namely, in regard to the nature of the delimitation of the spores, and of their plasma membranes, my observations indicate that in both zoosporangium and ascus the process of delimitation is practically the same, — a cleavage, — and that the plasma membranes are the same, and not in the one a fusion of astral rays. The point from which cleavage starts is a secondary matter. Within the Phycomycetes themselves there is great variability, the cleavages beginning from inner or outer plasma membranes or even from vacuoles. Whether or not the plasma membranes about the spores are entirely formed *de novo* in one case and not in the other, seems of little account, granted that they are the same and formed in essentially the same way.

The last objection has to do with epiplasm, its absence in the sporangium and its presence in the ascus. In the former the plasma membranes on either side of a cleft enclose a young spore, while in the latter but one of them performs that function, the other lining a cavity in the non-nucleated epiplasm. This epiplasm has been

looked upon as a natural and a necessary result of a *new* method of spore "cutting," and that it could not have arisen in the zoosporangium. That the *new* method referred to occurs in the ascus has not been borne out by my observations and that epiplasm might not have arisen in a zoosporangium is at least debatable. Granted nuclear control in sporogenesis, and a tendency towards a reduction in the number of spores without a corresponding and synchronous decrease in the protoplasm (a common feature in many Ascomycetes), and add to this, if need be, the acquirement of a precocious habit of spore-forming (especially if this means that the young spores depend to a large extent upon the contents of the spore sac, as is the case in asci) then epiplasm might very naturally be expected to follow in the zoosporangium, and without a change in the method of spore delimitation.

On the whole it does not appear incompatible with the facts to suppose that the homology of a Saprolegniineous zoosporangium with an ascus is at least a possibility, if, of course, there is no ontogenetical disqualification. As to that, if the Ascomycetes have sprung from the Oomycetes, then the regular alternation in the production of sexual and asexual organs exhibited by them has probably come about by the production, in certain transitional Oomycetes, of sexual organs only on the mycelia growing from the zoospores, their zoosporangia being suppressed or transformed into conidia, and the production of zoosporangia only by the mycelia growing from fertilized eggs. The latter zoosporangia have become asci.

It should be stated here that Juel (:**02a**) and Barker (:**03**) have recently expressed the view that the ancestor of the ascus is an Oomycetous zoosporangium. They reach their results, however, by a method that should be used only with great caution. They ground their conclusion on an analogy between spore-forming in the ascus and the oogenesis of the Oomycetes. The *raison d'être* of such an argument is based upon the theory that oogonia and antheridia are derivatives of algal gametangia, and that in the lower algae the gametangia are indistinguishable from and probably homologous with zoosporangia.

For the last two suggestions concerning the origin of the ascus there is little more to be said on the basis of the phenomena of sporogenesis. If the ascus could have been developed from the

Saprolegniineous zoosporangium, then it seems unnecessary to regard it as a new feature. As to its origin from a carpospore there are few known facts on which to base a conclusion, and such as are known are to the effect that the carpospores are uninucleate from the first, and continue to be so to the end.

Of the four suggestions offered, the facts of spore formation that have been adduced up to the present, seem, in the opinion of the writer, to favor the one that homologizes the ascus with the Oomycetous zoosporangium. If established, it is a strong argument in support of the theory that the Ascomycetes have been derived from the Oomycetes. Such a conclusion, however, can scarcely be considered as final, for as was indicated in the opening paragraph of this section, the phenomena of sexuality are equally important in determining the origin of the group, and deserve equal consideration. It is outside the purpose of the present paper to discuss that side of the subject, though it may be added that De Bary on the basis of sexuality arrived at the conclusion that there is a probability of the phylogenetic relationship of the Ascomycetes with some such Phycomycetous group as the Peronosporineae or Saprolegniineae.

Summary.

1. The asci bud out from the penultimate cells of the ascogenous hyphae in some forms, in others from the terminal cells, and in a few species apparently from any cell.

2. In every case that was definitely determined, the uninucleated stage is preceded by a fusion of two nuclei within the ascus. The conjugating elements, though not sisters, may sometimes be the daughters of sister nuclei.

3. The age and development of the asci at the time of fusion vary considerably in different species, and to some extent, though within narrower limits, in the same plant.

4. Extranuclear bodies or granules, evidently of nutritive matter, the staining reactions of which are very similar to those of the nucleolus, are formed in the young asci in the neighborhood of the nuclei, and probably through their activity. They disappear before the spores are matured and in many forms during the first spindle stage. Similar bodies frequently occur in the ripe spores.

5. The spindles are of intranuclear origin, but the centrosomes and asters with which they are typically associated are of extranuclear origin. The wall of the nucleus usually persists until a late anaphase stage, though exceptionally as in *Neotiella albocincta* it disappears before the equatorial-plate stage is passed.

6. The chromosomes vary for different species from four to eight in number. In Neotiella they are comparatively large and U-shaped.

7. Directly following the last mitosis in the ascus, and before the daughter nuclei pass into a resting condition, the spores are delimited and a plasma membrane is organized about them. The phenomena characteristic of this period are as follows: (1) an elongation of the spindle fibers bringing the daughter nuclei to the periphery of the mass of dense protoplasm in which they lie, and their centrosomes into close relations with the enveloping plasma membranes; (2) the delimitation of the spore plasm about each nucleus by the differentiation of a limiting layer of hyaline or finely granular protoplasm that begins adjacent to the centrosome and continues progressively until completed at the opposite pole; (3) the formation of a nuclear wall, the time of the appearance of which is variable for different species and bears no relation to the delimitation of the spore plasm; (4) the elongation of the nucleus after it has acquired a wall, resulting in a pear or beaked form with the centrosome at the end of the beak; (5) the organization of a plasma membrane about the spore plasm and another opposed to it lining the cavity in the epiplasm. These are formed on the site of the limiting layer and are probably preceded by a cleavage in the latter. The cleavage and organization of the membranes begin at the centrosome end of the spore and progress continuously to the opposite pole. (6) Retraction of the nuclear beak then follows and the nucleus assumes its regular spherical shape. Occasionally the centrosome, lying outside the nuclear wall but close against it, may be demonstrable for a short time after this re-formation. Very rarely, as in Hydnobolites, the astral rays persist up to this stage.

8. There is no evidence in the species studied that the astral rays fuse to form a membrane which cuts out the spore plasm and becomes the plasma membrane of the spore. They appear never to fuse, as is stated by Harper to be the case in Erisyphe and Lachnea, and the plasma membranes about the spores have quite a different origin.

9. After a period of growth and storage of reserve matter, an exospore is formed between the two opposed plasma membranes.

10. In the meantime the following changes may take place in the spores, according to the species to which they belong: (1) karyokinetic division of their nuclei without the formation of septa; (2) nuclear division followed by a corresponding septation; (3) the cutting off of an enucleated part of each spore, as in Podospora, by a septum, the organization of which is due to the direct action of the nucleus on the cytoplasm in its neighborhood.

11. The evidence points to the conclusion that while the ascus has probably not been derived from the sporangium of the Mucorineae, the phenomena of spore formation are not incompatible with the view that homologizes the ascus with the zoosporangium, nor with the view that the Ascomycetes have originated from some such Phycomycetous group as the Peronosporineae or Saprolegniineae, an affinity first suggested by De Bary on the basis of sexuality.

The investigations recorded in this paper were begun in the Laboratories of cryptogamic botany at Harvard university under the direction of Prof. Roland Thaxter, to whom I am under deep obligations for material and advice. My best thanks are also due Prof. W. G. Farlow and Dr. E. J. Durand for assistance in determination of species, Prof. E. C. Jeffrey for specimens of Tuber and Balsamia, and Professors C. B. Davenport and D. S. Johnson for facilities afforded in collecting material at the Biological station of the Brooklyn institute of arts and sciences at Cold Spring Harbor, L. I.

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EXPLANATION OF PLATES.

All drawings were made with the aid of a camera lucida. Fig. 69–79 are from cover-glass preparations, the rest from sections.

PLATE 7.

Fig. 1–16, *Hydnobolites* sp.

Fig. 1. Young uninucleated ascus. Differentiation of the contents into dense and vacuolated portions has already taken place. Very small extranuclear bodies lie just outside the nuclear wall.

Fig. 2. Oblique section of an older ascus. The dense protoplasm has become somewhat vacuolated. There are three large extranuclear bodies shown.

Fig. 3. Transverse section of upper part of a still older ascus. There are coarse fibrillar radiations out from the nucleus. The nucleus is surrounded by several small extranuclear bodies.

Fig. 4. Longitudinal section of a more advanced stage than in figure 3. Protoplasm finely reticular and delicately radiate about the nucleus. Extranuclear bodies scattered.

Fig. 5. Longitudinal section at time first aster appears. Extranuclear bodies occur at this stage near the periphery only.

Fig. 6. Equatorial-plate stage of first spindle.

Fig. 7. Same nucleus with spindle as in figure 6.

Fig. 8. First mitosis. Daughter chromosomes are formed.

Fig. 9. Early anaphase stage of first mitosis.

Fig. 10. Spindle elongating in anaphase stage of first mitosis. Chromosomes have reached the poles.

Fig. 11. First mitosis. Nuclear wall has disappeared. Protoplasm more coarsely reticulated except in the immediate neighborhood of the nucleus.

Fig. 12. Same spindle as in figure 11.

Fig. 13. End of first mitosis, showing extreme elongation of spindle and the vanishing nucleolus.

Fig. 14. One of the two daughter nuclei following first mitosis in resting condition. Single centrosome. The dense protoplasm is now uniformly and rather coarsely reticulated.

Fig. 15. Somewhat later stage than in figure 14. Protoplasm surrounding the nuclei begins to become finely reticulated and radiate. The fusion of vacuoles below is practically complete thus providing the dense protoplasm with a continuous plasma membrane.

Fig. 16. Two-spindle stage in longitudinal section of ascus.

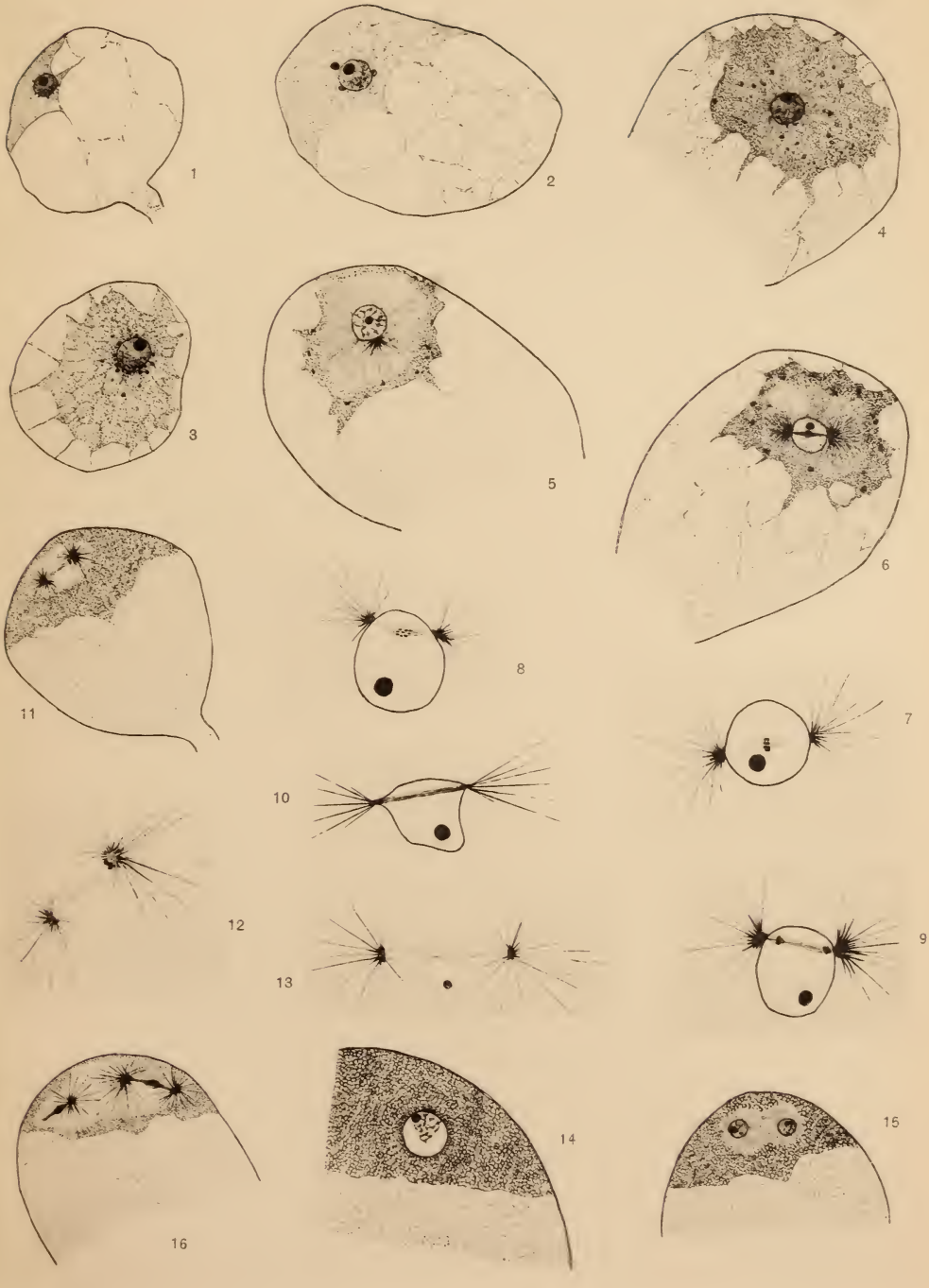

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PLATE 8.

Fig. 17–39, *Hydnobolites* sp.

Fig. 17. One of the spindles in figure 16. Late anaphase stage. Collapsed remains of the old nuclear wall still persists.

Fig. 18. Three of the four daughter nuclei in resting condition following second mitosis. Centrosomes are shown with two of them.

Fig. 19. Three of the four spindles of last mitosis.

Fig. 20. Spindle slightly younger than in figure 19, showing a mantle.

Fig. 21. Transverse section of top of ascus at end of last mitosis showing a polar view of five daughter nuclei.

Fig. 22. Intranuclear view of pole of the spindle of about the same age as in figure 19.

Fig. 23. Vertical section of nucleus of about the same age as in figure 21 or a little older. Spore plasm becoming differentiated in neighborhood of nucleus and the limiting layer beginning to form. Uppermost line represents wall of ascus.

Fig. 24. Older stage than in figure 23. Limiting layer not yet complete and nuclear wall hardly evident; dense protoplasm quite vacuolated.

Fig. 25. Polar view of about same age as in figure 23 or 24.

Fig. 26. Considerably later stage than in figure 24. Part of wall of ascus is drawn. The cylindrical nucleus is very obvious. The limiting layer is completed and already the plasma membranes are half completed.

Fig. 27. Later stage than in figure 26.

Fig. 28. Same age as in figure 27.

Fig. 29. The plasma membranes are nearly completed. They are somewhat separated from one another at upper pole.

Fig. 30. Plasma membranes completed.

Fig. 31. Same as in figure 30. Spore beginning to round out.

Fig. 32. As in figure 31 the centrosome is distinct. Both nucleus and spore show a lengthening.

Fig. 33. A polar view of about the same age as in figure 29 or 30. The astral rays have never exhibited a tendency to fuse laterally. The plasma membranes are separated.

Fig. 34. Retraction of nuclear beak under way.

Fig. 35. Nuclear beak entirely retracted.

Fig. 36. Astral rays have disappeared. Centrosome is only occasionally demonstrable after beak has been withdrawn. Plasma membranes of spore and epiplasm are considerably separated at one side of spore.

Fig. 37. Nucleus of spore in a resting condition. Directly after this the spore begins to elongate.

Fig. 38. Longitudinal section of ascus at about the stage represented in figure 29 showing relation of young spores to plasma membrane surrounding dense protoplasm.

Fig. 39. Transverse section of upper part of ascus at same age and for same purpose as in figure 38.

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PLATE 9.

Figs. 40–48, *Neotiella albocincta.*

Fig. 40. Mature one-nucleated stage of ascus.

Fig. 41. First spindle after cleavage of chromosomes. U-shaped chromosomes with very thick arms. Very fine astral rays.

Fig. 42. Somewhat older stage than represented in figure 41. Occasionally the long axis of the spindle is in a transverse plane, as it almost invariably is in *Urnula craterium.*

Fig. 43. Anaphase of first spindle stage. The arms of the chromosomes greatly lengthened.

Fig. 44. Equatorial-plate stage of second mitosis. Nuclear wall unusually persistent.

Fig. 45. Late anaphase stage at end of third mitosis. Limiting layer probably beginning to form.

Fig. 46. Nuclear walls re-formed and already growing towards center of ascus. The limiting layer is complete.

Fig. 47. Spore initials have grown somewhat and nuclear beaks have nearly reached their maximum length.

Fig. 48. Plasma membranes almost half completed.

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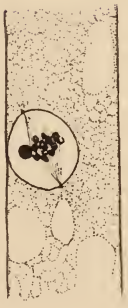

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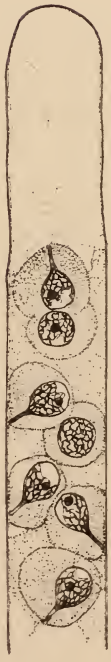

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PLATE 10.

Fig. 49–52, *Neotiella albocincta.*
Fig. 53–59, *Sordaria fimicola.*

Fig. 49. One of the spindles of last mitosis.
Fig. 50. Two spores in which the double plasma membranes are completed and shown slightly separated. The upper spore with two nuclei is a rare occurrence.
Fig. 51. Retraction of nuclear beak.
Fig. 52. Nucleus of a growing spore in resting condition.
Fig. 53. Four-nucleated stage of ascus at end of second mitosis. A centrosome outside of each nucleus.
Fig. 54. Last mitosis. Dense protoplasm quite vacuolated.
Fig. 55. Late anaphase stage of last mitosis. Spore plasm being differentiated and limiting layer forming.
Fig. 56. Limiting layer completed about each spore and nuclear wall formed.
Fig. 57. Plasma membrane is organized. Corresponding plasma membrane that covers cavity in epiplasm not represented in drawing.
Fig. 58. Karyokinesis in the spore.
Fig. 59. Binucleated spore.
Fig. 60. *Podospora zygospora.* Extranuclear bodies about the "fusion" nucleus.
Fig. 61. *Podospora curvula.* Extranuclear bodies as in figure 60.
Fig. 62. *P. curvula.* Nucleus has moved down to entrance of tail preparatory to forming a septum.
Fig. 63. *P. curvula.* Septum has been formed and nucleus has retired.

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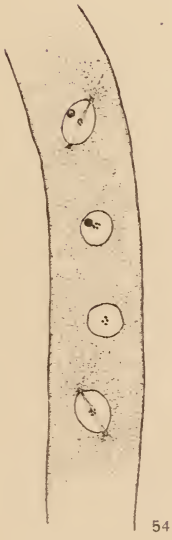
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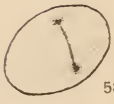
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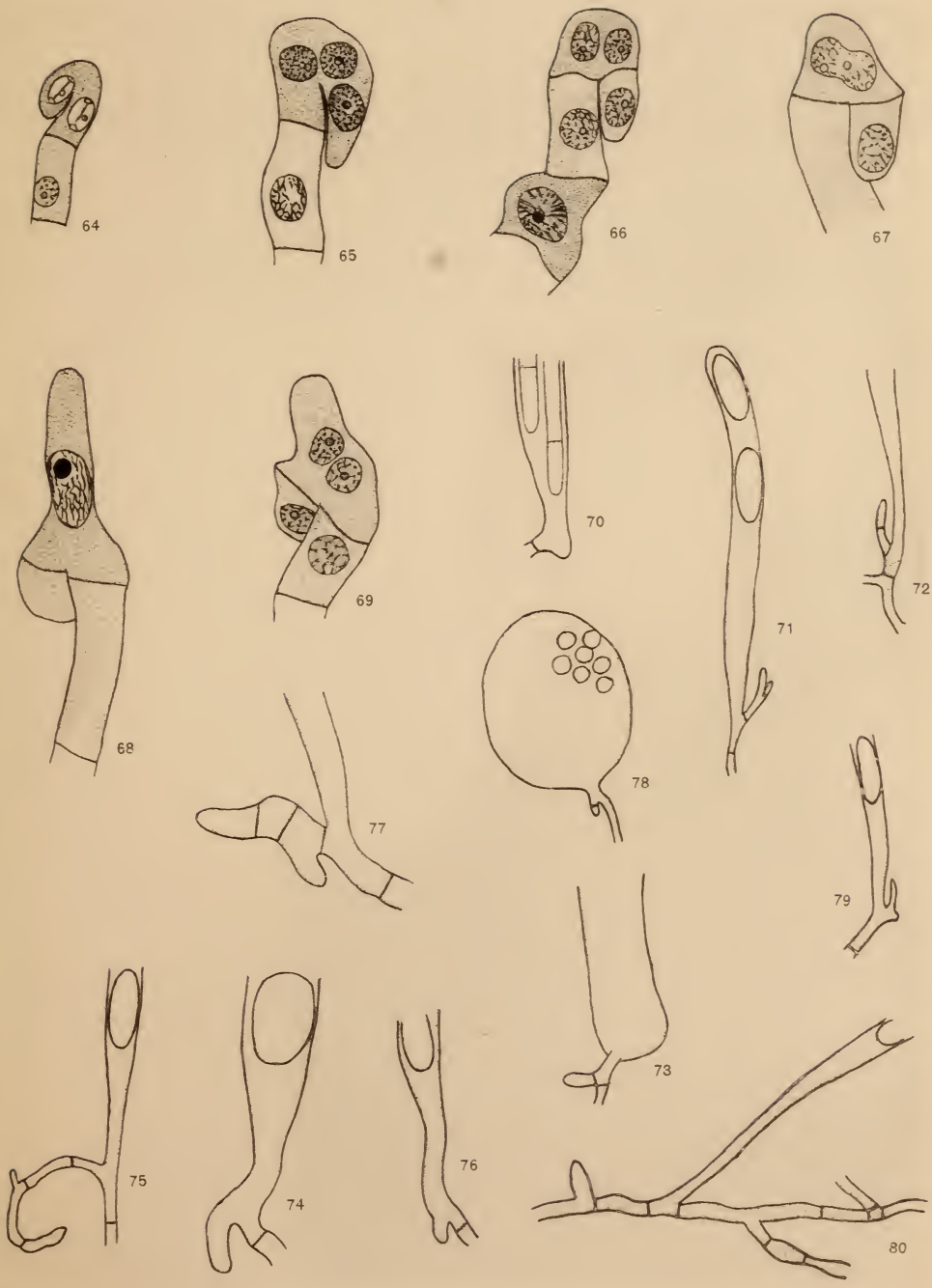
PLATE 11.

Fig. 64, *Peziza* sp.
Fig. 65–69, *Neotiella albocincta.*

Fig. 64. Nuclear division in hooked terminal cell of an ascogenous hypha prior to origin of ascus.
Fig. 65. One of the daughter nuclei of later stage than represented in figure 64 cut off by a septum from the rest.
Fig. 66. A second septum formed cutting off a nucleus and tip of hypha. The proximal cell seems to be preparing to form a new "hook."
Fig. 67. Fusion of nuclei in young ascus.
Fig. 68. Young ascus showing fusion nucleus.
Fig. 69. Fusion not yet effected, though ascus is growing out,— a frequent occurrence.
Fig. 70. Base of ascus of *Leptoglossum* sp.
Fig. 71. Base of an ascus of *Verpa bohemica.*
Fig. 72. Base of an ascus of *Verpa bohemica.*
Fig. 73. Base of an ascus of *Morchella esculenta.*
Fig. 74. Base of an ascus of *Genea hispidula.*
Fig. 75. Base of an ascus of *Verpa bohemica.*
Fig. 76. Base of an ascus of *Sordaria fimicola.*
Fig. 77. Base of an ascus of *Verpa bohemica.*
Fig. 78. Ascus of *Hydnobolites* sp.
Fig. 79. *Verpa bohemica*; showing base of ascus and end of ascogenous hypha.
Fig. 80. *Anixia spadicea*; showing base of ascus and ascogenous hypha.



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